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MODIFICATION OF AN ECOTOXICOLOGICAL RATING TO BIOASSESS SMALL ACID MINE DRAINAGE–IMPACTED WATERSHEDS EXCLUSIVE OF BENTHIC MACROINVERTEBRATE ANALYSIS

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Abstract—The modified ecotoxicological rating (METR) approach to synthesizing integrative bioassessment data into a single number ranging from 0 to 100 was found to differentiate between multiple levels of impacts from acid mine drainage (AMD). Our objective was to develop a more cost-effective and time-efficient bioassessment technique than previously used in other large scale ecotoxicological ratings (ETRs) by minimizing the number of parameters required to rank stations to only those most descriptive of the benthic macroinvertebrate community responses to AMD. Nineteen physical, chemical, toxicological, and ecological measurements were made at 38 stations in two adjacent watersheds. The most descriptive parameters were selected through multiple linear regression analysis, bivariate correlation analysis, and one-way analysis of variance. We found that habitat assessment, 30-d in situ Asian clam survival, mean conductivity, and mean total water column concentration of aluminum and manganese were the most descriptive parameters. The METR constructed from these parameters was equally effective at differentiating stations as were two previous published ETRs that incorporated up to 10 parameters, including benthic macroinvertebrate indices. When the METR was applied to a new watershed, the scores were significantly correlated with benthic macroinvertebrate indices for those stations.

Keywords—Acid mine drainage Rapid integrative bioassessments Ecotoxicological rating system

INTRODUCTION

Large-scale restoration efforts utilizing integrative bioassessment techniques to locate and rank contaminated areas of watersheds require intense sampling regimes [1–4]. For example, in the Leading Creek Ecological Enhancement Plan, more than 150 bioassessment parameters were measured on multiple occasions at 29 stations for five years [5]. During an initial assessment of the impacts of acid mine drainage (AMD) upon the Powell River (Lee County, southwestern Virginia, USA), 44 parameters were measured at 38 stations [6]. Recently, the U.S. Army Corps of Engineers in conjunction with the Virginia Department of Mines, Minerals, and Energy, Division of Mine Land Reclamation (Big Stone Gap, VA, USA), has begun a large-scale reconnaissance of AMD impacts upon the Powell River watershed. Because of the scale of the Powell River Ecosystem Restoration Project, projected to remediate numerous sites throughout the watershed including 11 subwatersheds of the North Fork of the Powell River (NFP River), those involved are interested in utilizing only the most descriptive and efficient bioassessment techniques available.

Integrative bioassessment approaches that incorporate both field and laboratory techniques, such as the sediment quality triad, have become increasingly popular [7–12]; a number of researchers have conducted assessments of heavy metal–impacted watersheds by comparing chemical, physical, and biological data to describe the environmental condition of the associated aquatic ecosystem [1,13–17]. Many of these studies used a sediment quality triad and weight-of-evidence approach to determine which stations were impacted and to provide insight toward causality of the observed impacts [18–20]. Often, these types of studies summarize integrative data with multivariate statistics and utilize a tabular decision matrix or triangular plot to categorize station groups relative to their environmental integrity in a visual manor easily understood by nonscientists [1,8,18,21–23]. However, summarizing data in this way limits the ability to statistically distinguish different levels of impact (i.e., heavily vs slightly impaired).

The ecotoxicological rating (ETR) system was designed to summarize abiotic and biotic parameters into a single value and to allow both rank ordering of individual stations according to relative impact and statistical comparisons between station groups. The ETR is conceptualized to work as an academic grading scale (0–100), rating reference stations with As (90–100) and Bs (80–89) and impacted stations with Cs (70–80), Ds (60–70), and failures (F ≤ 60). Two subwatersheds within the NFP River drainage have been assessed by the ETR approach, effectively differentiating between multiple levels of AMD impacts (i.e., acidic vs circumneutral AMD) [13,16]. These investigations employed more than 20 bioassessment parameters, including water and sediment chemistry and metals, habitat assessments, laboratory sediment and water column toxicity testing, in situ toxicity testing, and benthic macroinvertebrate collection and analysis, including percent Ephemeroptera abundance and Ephemeroptera–Plecoptera–Tricoptera (EPT) and taxon richness indices. Because the ETR is precise enough to differentiate between multiple levels of impact, has a quantitative basis for ranking the environmental integrity of stations, and is easily understood, the U.S. Army Corps of Engineers has adopted the ETR approach for use in further AMD remediation efforts within the Powell River watershed.

A common attribute of the previous ETR assessments, and most other ecological impact investigations, is the collection and analysis of the resident benthic macroinvertebrate com-

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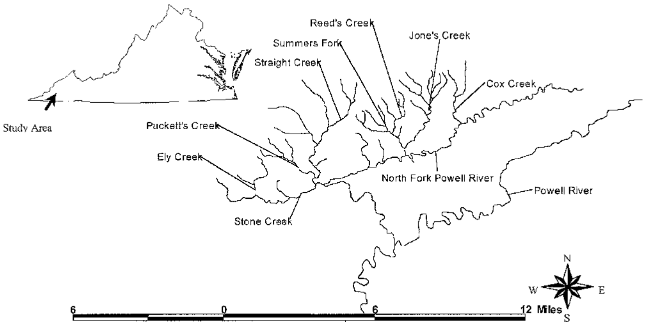

Fig. 1. Map of the North Fork of the Powell River (Lee County, VA, USA) and associated subwatersheds investigated in the current study.

munities. Valuable information can be derived from analyzing these benthic communities because they act as a continuous biological monitoring system, describing impacts in time frames far beyond a snapshot-sampling event of water chemistry or acute toxicity. However, benthic macroinvertebrate collection and analysis is a time-consuming and costly process [23]. Therefore, the objective of the present study was to develop a more cost-effective, time-efficient ETR approach specific to the NFP River AMD impacts by utilizing only a few highly descriptive parameters and eliminating the need for benthic macroinvertebrate surveys.

MATERIALS AND METHODS

Watershed characterization

Throughout the NFP River watershed the same geological formation, the Upper Mason coal seam has been heavily mined and is the primary source of AMD. Discharge from the Upper Mason coal seam empties into two adjacent tributaries of the NFP River, Ely Creek and Puckett's Creek (Fig. 1). These two tributaries share other similarities, such as area (6.0 and 7.4 km^2, respectively), mean pH range (2.9–7.7 and 2.9–8.0, respectively), and number of AMD seeps (five and four, respectively; Table 1). Acid mine drainage seeps contribute most of the water draining from the predominately rural, reclaimed lands that encompass the Ely Creek and Puckett's Creek watersheds. These similarities make the Ely Creek and Puckett's Creek systems (hereafter referred to as the paired system) ideal for investigating which bioassessment techniques best describe responses of benthic macroinvertebrate communities to AMD impacts from the Upper Mason coal seam, while accounting for the variability in community responses to other environmental differences between watersheds.

To validate the new modified ecotoxicological rating (METR), which was developed with data from the paired system, analysis of a third tributary impacted by AMD from the Upper Mason Coal Seam and within the NFP River watershed was necessary. Reed's Creek is a relatively larger system as compared to Ely Creek or Puckett's Creek (11.8 vs 6.0 and 7.4 km^2) but has somewhat lower total discharge characteristics (Table 1 and Fig. 1). Although Reed's Creek is impacted by more AMD seeps (19 areas of concentrated seepage), these seeps seem to cause less average toxicity (82% in situ Asian clam survival and 87% *Ceriodaphnia dubia* survival, $n = 15$ stations) at stations throughout the watershed, as compared to the seeps impacting Ely Creek ($n = 20$) and Puckett's Creek ($n = 21$) (44 and 53% in situ Asian clam survival, 59 and 62% *C. dubia* survival). The lesser degree of toxicity is thought to be a product of the diffuse and relatively neutral pH (mean pH values ranging from 5.5 to 7.2) of the seepage impacting Reed's Creek. The watershed characteristics of Reed's Creek provide an opportunity to test the effectiveness of the METR in a watershed with a different level of AMD impact.

Sample stations and ETR groups

Samples were collected in Ely Creek (January 1997–March 1997), Puckett's Creek (October 1997–July 1998), and Reed's Creek (December 1999–November 2000) over a four-year period. A total of 53 sampling stations were selected, with 16 stations in Reed's Creek, 20 in the Ely Creek study [13], and 21 in the Puckett's Creek study [16], with 4 overlapping stations between the latter two studies. Mean values were determined for all parameters that were measured at overlapping stations.

Each station was categorized according to relative level of AMD input as determined by location within the watershed and mean pH to facilitate statistical comparisons between the mean ETR scores of differentially impacted stations by analysis of variance (ANOVA) [16]. Group 1 sampling stations were categorized as being upstream of all known AMD inputs within the subwatersheds or in the NFP River. A second category (group 2) consisted of stations subjected to intermittent AMD input, which was designated as such based on the finding of wide pH ranges over time (e.g., 3.17–7.79), or being downstream of such a station but upstream of any continuous AMD input. Criteria for group 3 stations consisted of stations continuously subjected to AMD input and having acidic mean pH values ≤ 4.5 (no stations in Reed's Creek met this criterion). Stations continuously subjected to AMD input but that had mean pH values > 4.5, were categorized as group 4 stations. These stations generally occurred in third-order streams and

Table 1. Comparison of watershed characteristics between Ely, Puckett's, and Reed's creeks (Lee County, VA, USA)

Parameter	Ely Creek	Puckett's Creek	Reed's Creek
Watershed area (km^2)	6.0	7.4	11.8
Mean flow range (million gallons/d)	85–3,110	72–1,588	0.29–1,343
Mean pH range	2.9–7.7	2.9–8.0	5.5–7.2
Mean conductivity range (μmhos/cm)	50–2,420	65–1,600	148–710
No. AMD seeps[a]	5	4	19
Mean in situ Asian clam survival[b]	44	59	82
Mean *Ceriodaphnia dubia* survival[b]	53	62	87

[a] AMD = acid mine drainage.
[b] Describes the average survival of the test organisms at all stations within the watershed.

are referred to as being neutralized AMD stations rather than neutral mine drainage stations. Group 5 stations occurred in all three watersheds, and generally were found in fourth-order streams, but fit the criteria that they had one additional level of dilution downstream of group 4 stations.

Sample collection

Detailed descriptions of the sample collection and analysis methods for the Ely Creek and Puckett's Creek studies can be found in Cherry et al. [13] and Soucek et al. [16]. All data collected in Reed's Creek followed the procedures outlined in the two previous studies with the following exceptions. The lower detection limits for aluminum (Al) and manganese (Mn) in water-column samples from Reed's Creek were 0.06 and 0.024 mg/L, respectively. Habitat assessments by means of the U.S. Environmental Protection Agency rapid bioassessment protocols [24] were conducted at all stations as part of the original Ely Creek and Puckett's Creek studies; however, habitat assessments were not utilized as part of the Ely or Puckett's ETRs. Habitat assessments conducted in Reed's Creek followed the U.S. Environmental Protection Agency rapid bioassessment protocols [25]. Data transformations included a $\log(x + 1)$ transformation of all toxicological data to improve normality and homogeneity of variance, because many values were zeros.

Ecotoxicological parameter selection procedures

Three approaches were used in the parameter selection that produced the ranking systems resulting in the original Ely Creek and Puckett's Creek ETRs, and the new METR scores for Ely Creek and Puckett's Creek. Best professional judgment was used for parameter selection to develop the original Ely Creek ETR, as described in Cherry et al. [13]. The ETR parameter selection used in the original Puckett's Creek assessment utilized the chemical, ecological, and toxicological parameters that produced the largest statistical differences between station groups [16]. To produce the METR, multiple linear regression analysis was used to select parameters that best described benthic macroinvertebrate community structure as it changed relative to AMD impact, by using only chemical, physical, and toxicological parameters to develop ETR scores.

Statistical analyses performed to develop the METR were conducted with JMP IN® [26] software to select the chemical, physical, and or toxicological parameters (independent variables) that best correlated with the four selected benthic macroinvertebrate indices (total taxon richness, percent Ephemeroptera, EPT richness, and EPT abundance). Step-wise multiple linear regression analysis procedures were used to select an independent variable or variables (i.e., pH and sedimentary Al) that described each of the four dependent variables (i.e., benthic macroinvertebrate indices) with model significance determined at the $\alpha \leq 0.05$ level. Those independent variables selected in the step-wise procedures were then used in bivariate analyses to calculate correlation coefficients (r) with the dependent variables. Then, means of the absolute values of correlation coefficients were calculated between selected chemical, physical, and toxicological parameters and benthic macroinvertebrate indices, for the Ely Creek and Puckett's Creek data sets. To determine differences in the mean correlations an ANOVA and post hoc Tukey's t test with model significance at the $\alpha \leq 0.05$ levels were performed. Those independent variables found to have significantly larger average correlation coefficients than the others were selected to construct the METR scores for the stations in Ely Creek and Puckett's Creek.

Construction of ecotoxicological scores

Transformation of integrative data into ETR values for the METR followed Soucek et al. [16], except for water column metals. All chemical parameters were transformed into a percentage of the highest value measured in the subwatershed, and then subtracted from one, (1 − [value measured at a station/highest value measured in subwatershed]). This procedure creates values ranging from 0 to 1, giving stations with high chemical concentrations (e.g., sedimentary Al) values closer to 0 than stations with relatively low levels of contamination. A similar procedure often used with sediment quality triad studies, commonly referred to as ratio-to-reference, is described in Chapman [27] and DelValls et al. [21]. To create actual ETR scores each integrative parameter selected for use in the METR was transformed to a numeric unit ranging from 0 to 1; these were averaged together and then multiplied by 100. This procedure assumes each parameter contributes equal weight to the final score and results in a value ranging from 1 to 100. Higher scores are indicative of environmental conditions that are less impacted as compared to stations with lower scores. For example, an unimpacted reference station might have an ETR score of 95, as compared to an impacted station with an ETR score of 52.

Comparison of ETR parameter selection procedures

Comparisons between the original Ely Creek and Puckett's Creek ETRs and the METR were used to test how parameter selection procedures affect ETR performance in differentiating between station group categories (groups 1–5). Two separate ETR scores were developed for all stations in Ely Creek by using the original Ely Creek ETR and the METR. Two separate ETR scores also were developed for each station in Puckett's Creek, by using the original Puckett's Creek ETR and the METR. Mean station group ETR scores were compared between the original Ely Creek and the METR, and between the original Puckett's Creek and the METR using an ANOVA with model significance at the 0.05 level and a post hoc Tukey's t test ($\alpha \leq 0.05$).

Statistical analysis of METR validation

To validate the effectiveness of the METR at differentiating between station groups and capability of rank ordering stations in any AMD-impacted subwatersheds of the NFP River, the same ETR approach used in the Ely Creek and Puckett's Creek subwatersheds was applied to data collected in Reed's Creek. In addition, to validate that the METR was predictive of the benthic macroinvertebrate communities, correlation coefficients were developed between the same four benthic macroinvertebrate indices used to develop the METR, and the METR score for stations in Reed's Creek.

RESULTS

Selection of ETR parameters

Step-wise models produced by multiple linear regression analysis explained 70 to 95% of the variation in the dependent variables from station to station in Ely Creek and 55 to 68% of the variation in dependent variables between stations in Puckett's Creek (Table 2). Twelve of the 18 chemical, toxicological, and physical parameters were selected by one of the eight regression models at least once: *Chironomus tentans*

Table 2. Prediction equations for benthic macroinvertebrate community indices based on multiple linear regression analysis for Ely and Puckett's creeks (Lee County, VA, USA)[a]

Taxa richness	[b] $y = -23.54 + 0.38(\text{habitat})$	($R^2 = 0.74$, total $df = 19$, $p < 0.0001$)
	[c] $y = 24.14 + -0.01(\text{conductivity}) - 0.129(\text{sedimentary Zn})$	($R^2 = 0.55$, total $df = 20$, $p = 0.0007$)
% Ephemeroptera abundance	[b] $y = -85.74 + 0.001(\text{sedimentary Fe}) - 0.675(\text{sedimentary Zn}) + 1.38(\text{habitat})$	($R^2 = 0.70$, total $df = 19$, $p = 0.0002$)
	[c] $y = 60.14 - 0.0337(\text{conductivity}) - 1.76(\text{sedimentary Ni})$	($R^2 = 0.68$, total $df = 20$, $p < 0.0001$)
EPT[d] richness	[b] $y = -14.89 + 0.24(\text{habitat})$	($R^2 = 0.72$, total $df = 19$, $p < 0.0001$)
	[c] $y = 22.51 - 0.01(\text{conductivity}) - 4.66(\textit{Daphnia magna}\ \text{reproduction}^{e})$	($R^2 = 0.63$, total $df = 20$, $p = 0.0001$
EPT abundance	[b] $y = -189.68 + 14.04(\text{asian clam survival}) + 50.20(\textit{Chironomus tentans}\ \text{survival}) - 137.50(\textit{C. tentans}\ \text{weight}) + 11.80(\text{sedimentary Ni}) - 3.86(\text{sedimentary Zn}) + 7.80(\text{Al in } H_2O) - 18.32(\text{Mn in } H_2O) + 2.08(\text{Fe in } H_2O) + 2.54(\text{habitat})$	($R^2 = 0.95$, total $df = 19$, $p < 0.0001$)
	[c] $y = 61.53 - 148.93(\textit{D. magna}\ \text{reproduction}^{e}) - 6.19(\text{sedimentary Ni}) - 8.49(\text{Fe in } H_2O) + 4.63(\text{habitat})$	($R^2 = 0.65$, total $df = 20$, $p = 0.0015$)

[a] The best model for each index as determined by a step-wise selection procedure is shown. For each model, all variables contribute significantly to the overall model ($\alpha \leq 0.05$).
[b] Regression equation for Ely Creek.
[c] Regression equation for Puckett's Creek.
[d] EPT = Ephemeroptera–Plecoptera–Trichoptera.
[e] *Daphnia magna* reproduction (sediment toxicity endpoint) is shown as number of neonates (% of control).

survival, *C. tentans* weight, *Daphnia magna* reproduction, water column iron (Fe), habitat assessment, in situ Asian clam survival, water column Al, water column conductivity, water column Mn, sedimentary Fe, sedimentary nickel (Ni), and sedimentary zinc (Zn). Habitat assessment appeared in five of the eight multiple linear regression analysis models, whereas water column conductivity and sedimentary Ni and Zn were in three models. *Daphnia magna* reproduction and water column Fe occurred in two multiple linear regression analysis models, with the remaining five parameters contributing to only one model.

These 12 selected parameters then were subjected to bivariate correlation analysis with the four ecological parameters, and mean correlation coefficients were calculated for each parameter and analyzed with an ANOVA. This analysis indicated that habitat assessment was significantly more descriptive of the four ecological indices than the other parameters, with a mean r value of 0.61 ($p < 0.0001$; Table 3). The other 11 parameters produced mean r values ranging from 0.15 to 0.52. In situ Asian clam survival (mean r value = 0.52), water column conductivity (mean r value = 0.48), Mn (mean r value = 0.47), and Al (mean r value = 0.45), all were found not to be statistically lower than habitat assessment. The r values of the other seven parameters ranged from 0.15 to 0.38, but were found to be statistically different from habitat assessment. All parameters found to be similar to habitat assessment were used to construct the METR (Table 4).

Table 3. Mean (± standard deviation) absolute value of bivariate correlation coefficients (r) for comparisons of the 12 parameters selected in multiple linear regression analysis with four benthic macroinvertebrate indices in Ely and Puckett's creeks (Lee County, VA, USA)

Ecotoxicological rating parameter	Mean correlation coefficients
Habitat	0.61 ± 0.24 A
Asian clam survival	0.52 ± 0.10 AB
Conductivity	0.48 ± 0.14 AB
Mn in H_2O	0.47 ± 0.16 AB
Al in H_2O	0.45 ± 0.11 ABC
Fe in H_2O	0.38 ± 0.10 BCD
Chironomus tentans weight	0.25 ± 0.06 CDE
C. tentans survival	0.24 ± 0.03 CDE
Sedimentary Zn	0.24 ± 0.13 CDE
Sedimentary Ni	0.23 ± 0.15 DE
Sedimentary Fe	0.19 ± 0.14 DE
Daphnia magna reproduction[b]	0.15 ± 0.08 E

[a] Means followed by the same uppercase letter are not significantly different, Tukey's t test results ($p < 0.0001$).
[b] *Daphnia magna* reproduction (sediment toxicity endpoint) is shown as number of neonates (% of control).

Two ETR scores were constructed for each station within Ely Creek and Puckett's Creek by using the original ETR designed for the respective subwatersheds and the METR as described above. Mean ETR scores for each station group were then calculated for each method and compared by ANOVA (Table 5). Significant differences ($p < 0.0001$) were observed among groups in all four ETRs. The original ETRs constructed for Ely Creek had the same significant differences between station groups as the METRs. In all cases, reference stations (group 1) had the highest average ETR score, with the two METR scores averaging higher ETR scores than the original ETR. In all cases again, group 3 stations had the lowest average ETR scores, with the original ETRs having the lower two average values. The original ETRs constructed for Ely Creek and Puckett's Creeks had the same significant differences between station groups as the METRs.

Validation of the METR

In the validation subwatershed (Reed's Creek) group 1 (reference) stations, the average METR score was 87.6 (Table 6). These stations were not found to be statistically different from group 5 stations (highest average METR score of 88.7), which are located one level of dilution downstream of group 4 stations. Intermittently acidic stations (group 2) averaged the lowest METR score, whereas group 4 stations (neutralized AMD stations averaged 70.2) were found not to be different from stations in groups 1, 2, and 5. The range of the average METR scores for stations within the Reed's Creek subwatershed was 33.3. When group 3 stations are excluded from the Ely Creek and Puckett's Creek ETRs for comparison (Ely Creek ETRs, original = 38.7, Reed's Creek METR = 35.5; Puckett's Creek ETRs, original = 26.3, Reed's Creek METR = 20.3), the Reed's Creek METRs average range of score is consistent with

Table 4. Parameters selected in respective ecotoxicological rating (ETR) systems[a]

Original Ely Creek ETR	Original Puckett's Creek ETR	METR	METR ranking procedure
Mean conductivity	Mean conductivity	Mean conductivity	1 − % of highest value
Asian clam in situ survival		Asian clam in situ survival	% of highest value
Al in H_2O		Al in H_2O	1 − % of highest value
Ceriodaphnia dubia survival[b]	*C. dubia* survival[b]		
Daphnia magna survival[c]	*D. magna* survival[c]		
% Ephemeroptera	% Ephemeroptera		
Taxa richness	EPT richness	Mn in H_2O	1 − % of highest value
Mean pH	Fe in H_2O	Habitat	% of highest value
Sedimentary Fe			
Chironomus tentans survival[c]			

[a] Parameters selected for the original Ely Creek ETR were based on best professional judgment. Parameters selected for the original Puckett's Creek ETR were based on sensitivity, multiple linear regression analysis, and correlation analysis. Parameters selected for the modified ecotoxicological rating (METR) were based on multiple linear regression analysis and correlation analysis with benthic macroinvertebrate indices, by selecting parameters most descriptive of those indices. A station's rankings were then averaged, and multiplied by 100 to result in the final ETR score. EPT = Ephemeroptera–Plecoptera–Trichoptera.
[b] Water column toxicity endpoint.
[c] Sediment toxicity endpoints.

those found in the paired watershed system (Table 5). All correlation coefficients between METR score and the four benthic macroinvertebrate indices were significant, with p values ≤ 0.01 and r values ranging from 0.62 for EPT abundance to 0.70 for percent Ephemeroptera (Table 7).

DISCUSSION

The results of the present study suggest that the METR approach synthesizes integrative data from AMD-impacted sampling stations into a single numerical index sensitive enough to statistically differentiate multiple levels of impact (i.e., upstream acidic vs neutral mine drainage). By utilizing benthic macroinvertebrate community indices to select parameters for the purpose of ranking stations, data from other subwatersheds (i.e., Ely and Puckett's creeks) can be used to develop a system that can rank stations in other subwatersheds relative to AMD impacts and retain the ability to describe the benthic community at those stations (i.e., Reed's Creek). Because the METR utilizes fewer, more descriptive parameters then past ETRs, and can be applied to new subwatersheds of the NFP River without benthic macroinvertebrate analysis, it is a more time- and cost-efficient ranking system than previous ranking approaches used in the NFP River watershed (i.e., original ETRs for Ely Creek and Puckett's Creek).

Chapman [18,28] and Chapman et al. [29] established that summary indices should be avoided when using sediment quality triad studies intended to rank impaired stations because summary indices do not effectively distinguish intermediate impacts [1,10]. Both the original ETR and METR constructed for Ely Crrek and Puckett's Creek differentiated reference (group 1) and recovery stations (group 5) from stations receiving both acutely toxic AMD inputs (group 3) and intermittently impacted stations (groups 2 and 4; Table 5). To further assess the resolution of the METR in systems of intermittent and intermediate impacts, a third more diffusely impacted subwatershed of the NFP River (Reed's Creek) was investigated with the METR. Reed's Creek is a system without acutely toxic AMD impairments, as demonstrated by the lack of group 3 stations (stations with mean acid pH) and greater mean percent survival of two test organisms (Asian clam and *C. dubia*) at all stations than in the paired watersheds (Table 1). However, when group 3 stations were removed for comparison, the mean range of METR scores between station groups in Reed's Creek (mean score range = 33.3) is similar to or larger than the mean score ranges found in Ely Creek (35.5) or Puckett's Creek (20.3). Analysis of these data suggests that the METR has similar or improved resolution in ranking stations impacted by intermittent or intermediate AMD as compared to the past ETR studies.

The METR takes a novel approach to utilizing integrative

Table 5. Mean (± standard deviation) ecotoxicological ratings (ETR score) created for station group in Ely and Puckett's creeks (Lee County, VA, USA) as created by the modified ETR (METR) and the original ETR for that watershed[a]

Ely Creek ETRs			Puckett's Creek ETRs		
Station group[b]	METR scores	Original ETR scores	Station group[b]	METR scores	Original ETR scores
1 (n = 5)	94.5 ± 7.2 A	79.5 ± 11.3 A	1 (n = 7)	93.8 ± 2.3 A	80.1 ± 11.1 A
2 (n = 3)	59.0 ± 12.3 BC	40.8 ± 7.2 BC	2 (n = 3)	73.5 ± 11.8 B	54.9 ± 4.7 B
3 (n = 3)	37.8 ± 12.7 C	20.6 ± 14.4 C	3 (n = 4)	30.1 ± 16.4 C	15.9 ± 14.7 C
4 (n = 5)	65.9 ± 11.2 B	49.5 ± 11.6 B	4 (n = 3)	73.6 ± 9.1 B	53.8 ± 4.3 B
5 (n = 4)	82.2 ± 8.2 AB	63.8 ± 11.6 AB	5 (n = 4)	86.6 ± 3.6 AB	69.8 ± 6.0 AB

[a] Means followed by the same uppercase letter are not significantly different, Tukey's t test results ($p < 0.0001$).
[b] 1 = no acid mine drainage (AMD) impact; 2 = intermittent AMD impact; 3 = acidic AMD stations; 4 = neutral AMD impact; 5 = receiving system stations.

Table 6. Mean (± standard deviation) modified ecotoxicologic rating (METR) of each station group in Reed's Creek (Lee County, VA, USA)[a]

Station group[b]	METR scores
1 ($n = 3$)	87.6 ± 2.7 A
2 ($n = 3$)	55.4 ± 21.5 B
3 ($n = 0$)	NA
4 ($n = 7$)	70.2 ± 9.7 AB
5 ($n = 2$)	88.7 ± 2.5 A

[a] Means followed by the same uppercase letter are not significantly different, Tukey's *t* test results ($p < 0.0001$).
[b] 1 = no acid mine drainage (AMD) impact; 2 = intermittent AMD impact; 3 = acidic AMD stations; 4 = neutral AMD impact; 5 = receiving system stations. NA = not available (no stations met group 3 criteria).

data often not associated with triad-based ranking studies. Historically, information regarding the environmental integrity of impacted stations is pooled together from the ecological, toxicological, and chemical aspects of the sediment quality triad, assuming that utilizing all three data types will establish a chain of evidence of contamination or biological impairment by a stressor. The result is a triad of information including data from multiple levels of biological organization, both field and laboratory validated, and an approach that results in a chain of evidence that demonstrates environmental contamination and biological impairment. Benthic macroinvertebrate analyses alone contribute the largest extent of this information and by design, the METR approach recognizes this utility and uses these ecological indices as the focus of parameter selection. Correlations between the chemical and toxicological parameters and the ecological parameters (benthic indices) can establish a chain of evidence between those parameters most correlated with changes in the resident benthic communities. For example, habitat assessment and Al in the water column, as measured at each station, explained an average of 78 and 69%, respectively, of the variation in the four benthic indices analyzed; however, sedimentary Ni and Fe only explained 48 and 44% of the variation, on average (Table 3). This might suggest that habitat degradation and high levels of Al in the water column significantly contribute to the observed resident benthic infaunal impairment relative to reference areas. With this approach, resource managers might focus efforts on remediation techniques that improve in-stream habitat and decrease Al levels in the water column in Ely and Puckett's creeks to more efficiently remediate these systems.

In the development of a summary index such as the ETR, the parameters selected for use in the ranking procedures must be evaluated according to the weight or descriptive power each parameter contributes to the ranking. The METR approach also provides logic to how much weight each parameter is given and how much it contributes to the ranking system. By utilizing those parameters that have statistically similar descriptive power of the resident community structure, equal distribution of the descriptive nature of each parameter is predetermined and all selected parameters are treated equally.

Table 7. Correlation coefficients (*r*) for comparisons of mean modified ecotoxicologic ratings with benthic macroinvertebrate indices of Reed's Creek (Lee County, VA, USA)

Benthic macroinvertebrate indices	Correlation coefficients
Taxa richness	0.67 ($p = 0.006$)
% Ephemeroptera abundance	0.70 ($p = 0.004$)
EPT richness[a]	0.67 ($p = 0.007$)
EPT abundance	0.62 ($p = 0.010$)

[a] EPT = Ephemeroptera–Plecoptera–Trichoptera.

The use of a sensitive 30-d in situ test with Asian clams contributes significantly to capabilities of the METR (Table 3). The significance of the contribution of this parameter to the METR is ultimately found in the bridge it builds between changes in the resident benthic macroinvertebrate community structure as impacted by AMD and the laboratory-measured contaminant levels and toxicity [29,30]. Through the conduction of benthic macroinvertebrate surveys during or immediately after the 30-d in situ Asian clam test, differences in the community structure found at stations can be associated with the survival of Asian clams at those stations during that time period. By cojoining water column and sediment chemical analysis with these field measurements, a reasonable argument can be made for causality if necessary.

The specific bioassessment techniques incorporated into the METR as developed in the NFP River watershed are not necessarily appropriate for use in other watersheds or other bioassessment scenarios. Rather, the METR as an approach is valuable when assessing which bioassessment techniques provide the greatest descriptive power before implementation in large-scale ecosystem assessments. This approach should not be restricted to lotic systems, but could be used in both marine and lentic environments if historical data are available in the areas of interest. The METR approach need not be restricted to the bioassessment techniques used in these and past ETR studies, but could be implemented on any historical integrative bioassessment data set that meets the primary assumptions of regression.

This study demonstrates how the METR approach can be used to develop a numerical summary index that synthesizes integrative data and ranks stations relative to how the resident benthic macroinvertebrate community structure is impacted by AMD. The METR is a summary index sensitive enough to differentiate between multiple levels of environmental impacts from AMD and is an approach that results in a time- and cost-efficient bioassessment that is easily understood by nonscientists. The ETR approaches currently are being utilized by the U.S. Army Corps of Engineers to prioritize AMD-impacted stations in subwatersheds of the NFP River for ecological restoration.

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